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EFFECT OF LEGUME AND GRASS RESIDUES
ON ROOT NECROSIS BY *PYTHIUM GRAMINICOLUM*

by

Wilbur Leonard Staudinger

A Dissertation Submitted to the
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Major Subject: Plant Pathology

Approved:

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In Charge of Major Work

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Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1961

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INTRODUCTION

The living root systems of crop plants might conceivably increase, decrease, or have no effect on the disease inciting potential of pathogen-infested soil. Any effect by a particular crop on a specific pathogen would probably depend upon susceptibility of the crop and on the abundance of the pathogen in the soil when the crop roots first permeated it.

Pythium graminicolum Subr. is a root infecting fungus that probably does not exist saprophytically in the soil. The persistence of this fungus is probably in the form of oospores since the vegetative stage is inhibited by fungistatic properties of cropped field soils (Harper 1960).

Unlike many of the sphaerosporangiate *Pythium* species, the host range of *P. graminicolum* is for the most part restricted to members of the Gramineae. Among Iowa crop plants corn and sorghum are susceptible to this fungus, whereas soybean (Knaphus 1960) and alfalfa* are not.

Previous investigations have indicated that various crop residues incorporated in soil may effectively reduce incidence of root disease. However, there is no reported information concerning the effect of crop residues on the disease inciting potential by soil-infesting members of the Pythiaceae.

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*Buchholtz, W. F., Ames, Iowa. Susceptibility of alfalfa to *P. graminicolum*. Private communication. 1961.

Two lines of investigation are reported in this dissertation. One involves the effect of the living root systems of two susceptible (corn and sorghum) and two nonsusceptible crops (soybean and alfalfa) on the *P. graminicolum* disease inciting potential. The other involves the effect of residues of the above crops on the *P. graminicolum* disease inciting potential of the soil to which the residues had been added.

LITERATURE REVIEW

Pythium graminicolum, a common root pathogen, was first described by Subramanian in 1928. Unlike many of the sphaerosporangiate Pythium species the host range of *P. graminicolum* is for the most part restricted to graminaceous hosts (Middleton 1943). *P. graminicolum* is most prevalent at a soil depth of three to six inches (Knaphus and Buchholtz 1958), and in nature rarely induces seed rotting and damping-off but probably first infects roots some time after emergence and plant growth have occurred (Buchholtz 1949, Gerhold 1947, Hampton and Buchholtz 1959, Ho, Meredith and Melhus 1941, Summers and Buchholtz 1958).

On the basis of frequency of recovery of *P. graminicolum* from field grown corn roots placed in nonnutrient agar, Hampton and Buchholtz (1959) found that infection occurred most frequently in early June. Thereafter, the abundance of this fungus in corn roots was associated positively with soil moisture and negatively with maximum soil temperature.

In greenhouse tests Hampton (1957) showed that nitrogen and phosphorous added to *P. graminicolum* infested soil had no effect on susceptibility of, or disease development in corn, wheat, oats and barley plants. Instead, the addition of fertilizer usually produced plants of lower weight as compared to those grown in nonfertilized infested soil.

The cause of crested wheatgrass stand failures in central

South Dakota was investigated by Buchholtz in 1948. Symptoms of wheatgrass plants in field soils involved seed rotting, wilting, defoliation, stunting and death of plants. P. graminicolum, Fusarium spp., P. debaryanum Hesse and Helminthosporium sativum Pammel, King and Bakke were isolated from wheatgrass roots placed in nonnutrient agar, and the role of the above organisms in wheatgrass seedling blight was investigated. By soil infestation experiments, Buchholtz found that P. graminicolum induced germination failure, stunting and wilting, P. debaryanum incited germination failure only and H. sativum caused seed-rotting and necrosis of young wheatgrass seedlings. Only P. graminicolum induced wilting and death of wheatgrass plants when soils supporting plants 5, 8, 12, 15, 22 and 28 days old were infested (Buchholtz 1949).

Knaphus and Buchholtz (1958) utilized crested wheatgrass as an indicator plant to determine the relative abundance of P. graminicolum in soil samples collected at different depths. Counts of diseased and healthy plants, and recovery of P. graminicolum from sections of wheatgrass roots placed in water agar were used as criteria for establishing the vertical distribution of this fungus.

Harper (1960) employed crested wheatgrass to establish the P. graminicolum disease inciting potential of infested soils. Crested wheatgrass seed were planted in soils two weeks after infestation. Seed germination failure, numbers of dead plants and recovery of _P_. _graminicolum_ from root isolations were used to establish the degree of soil colonization by this fungus.

Effects of organic amendments incorporated in soils predisposed to a high frequency of root infections have been investigated by many workers. Millard (1923) reported effective control of the potato scab disease by amending naturally infested field soil with grass cuttings. Comparatively recent field experiments in Canada have shown that green manurial amendments of soybean reduced scab disease, whereas no control was obtained from clover or rye residue treatments (Rouatt and Atkinson 1950).

In greenhouse and field tests Fellows (1929) reported biologic control of the take-all disease by soil amendments of chicken manure, horse manure, alfalfa, boiled barley and oat mixture, and potato flour. Clark (1939) supported Fellows' findings in that green alfalfa manure and chicken manure incorporated in naturally infested field soils effectively suppressed _Ophiobolus graminis_ Sacc. infection of wheat roots.

The effect of wheat, oat and barley straw incorporated in soil infested with _Ophiobolus graminis_, _Helminthosporium sativum_, and _Fusarium culmorum_ (W. G. Smith) Sacc. on the severity of disease on the basal parts of wheat seedlings was studied by Tyner (1940). Wheatstraw amendments were distinctly more favorable to disease development than oat or barley straw residues. The least disease occurred on roots of wheat grown in oat amended soils.

Tolmsoff and Young (1959) investigated the effect of various plant residues and inorganic nitrogen on potato wilt caused by Verticillium albo-atrum Reinke and Berthold. Barley and oat residues depressed disease development, whereas soybean, clover and alfalfa residues had no effect. At nine tons per acre of oats or barley residue, wilt suppression markedly increased with an increase in inorganic nitrogen.

Snyder et al. (1959) incorporated numerous plant residues in soil naturally infested with Fusarium solani f. phaseoli (Burk.) Snyd. and Hans., Rhizoctonia solani Kuhn and Thielaviopsis basicola Berk. Bean seed were planted in residue amended soils and 10 days later the severity of root infection recorded. Plant residues with high C:N ratios such as mature barley straw, wheat straw, corn stover and pine shavings decreased the incidence of disease, whereas green barley hay, soybean and alfalfa residues with low C:N ratios increased disease severity.

One of the earliest methods recommended to reduce the cotton root disease caused by Phymatotrichum omnivorum (Shear) Duggar in irrigated soils of the Southwestern United States was to incorporate green alfalfa or corral manure in infested soils two months prior to sowing cotton (King et al. 1934, King 1937). Jordan et al. (1939) reported suppression of cotton root rot following rotation and incorporation of sorghum stalks in naturally infested field soils. In addition, Jordan obtained root rot reduction when cotton stalks were turned under as soon as harvest had been completed instead of late in the winter as was the usual agricultural practice.

Hildebrand and West (1941) and West and Hildebrand (1941) in a study of the relative effects of various cover crops on strawberry root rot showed that the disease was almost completely controlled in a naturally infested root rot soil in which several crops of soybeans had been incorporated. Under a similar treatment with red clover the disease was severe.

Richardson (1942) investigated the effect of crop residues on corn root necrosis. Four successive crops of each of 12 host plants and four-crop rotations of different hosts were grown in naturally and artificially infested soils. Leafy succulent growth from each crop was incorporated with the soil in which it had grown and initial decomposition allowed to occur before the succeeding crop was sown. Sixteen days after incorporation of the last cover crop, corn was planted in each of the pots. The general effects of the cover crop residues follow: successive crops of soybeans resulted in complete disease suppression in both naturally and artificially infested soils. Oats, tomato and rape residue amended soils produced corn plants with only a few visible lesions. Somewhat more severe infection of corn roots occurred following wheat, alfalfa, buckwheat and sugar beet amendments, and red clover and rye treatments were conducive to general infection of the corn root system.

In summary, the modification of many soil-borne plant diseases through organic amendments has been studied. However, little information concerning the effect of organic amendments on root diseases incited specifically by the Pythiaceae has been reported. Since these fungi are important root pathogens, investigations of the effect of two legume and two grass crop residues on root infection by *Pythium graminicolum* were undertaken.

MATERIALS AND METHODS

Experiments reported here were essentially of two types. One involved collection of soil samples from treated field plots and determining their *Pythium graminicolum* disease inciting potential by greenhouse tests. The other involved assaying for *P. graminicolum* disease inciting potential in the field. In all experiments crested wheatgrass, *Agropyron cristatum* (L.) Beauv., was used as an indicator plant to delineate differences among disease inciting potentials of variously treated soils. A single lot of crested wheatgrass seed with a laboratory germination of 81 per cent was obtained from the Iowa State Seed Testing Laboratory and was used for all disease inciting potential tests.

During the 1960 and 1961 growing seasons soil samples were collected from field plots, taken to the greenhouse and assayed for their *P. graminicolum* disease inciting potential. Soil sampling procedures were as follows: a composite soil sample from five random locations in a treated field plot was placed in a plastic bag. Each plot was sampled as above and samples from individual plots were kept separate in subsequent tests. In 1960 the treatments consisted of growing crops in field plots, and soil samples were taken one inch diagonally from the cover crop stems. Soils thus collected contained roots of the cover crops. In 1961 the field plot treatments were crop residue amendments. In each case, the sampling tool was a small trowel scraped free of adhering soil particles and washed in five per cent formaline solution between plot samplings.

Immediately following each collection, the soil samples were taken to the greenhouse, individually homogenized, screened through an eight mesh screen and placed in steamed clay pots. Crested wheatgrass was sown in soil thus treated and three weeks later the numbers of surviving and surviving diseased plants were recorded. Surviving plants were removed from pots and the soil washed from the roots with a fine spray of water.

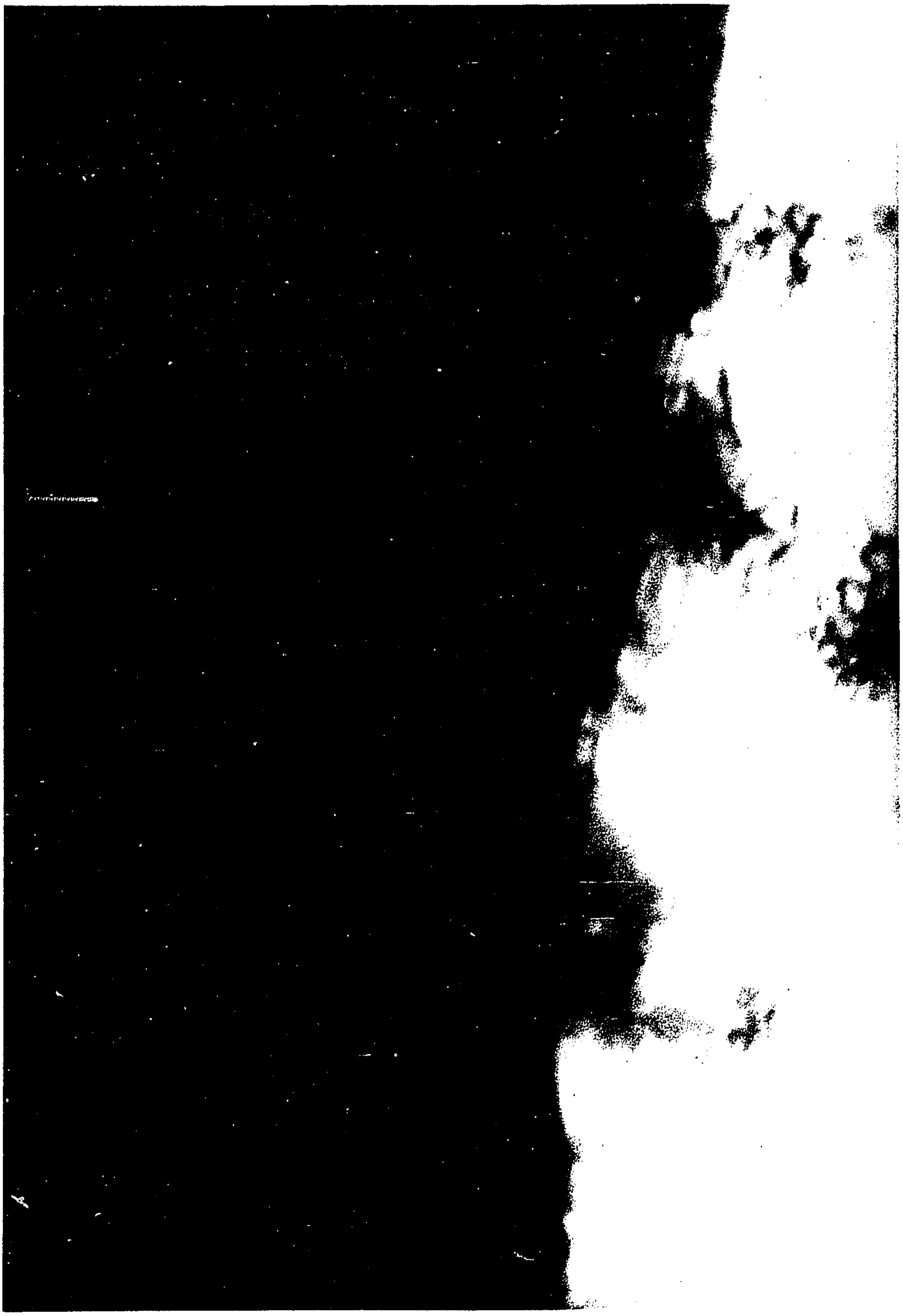
Isolations from roots of randomly selected crested wheatgrass plants grown in variously treated soils were made by placing 0.5 to 1.0 cm root sections in petri plates containing about 15 ml of 1.5 per cent water agar. Four days after making the isolations the water agar plates were examined for the presence of nematosporangia typical of _P. graminicolum_ produced on hyphae obviously attached to an individual root-piece (Fig. 1). The percentage recovery of _P. graminicolum_ was recorded. Statistical analyses were made of arcsin transformations of percentage data. Wheatgrass plants not used in the above isolation procedures were dried at 100 ^{o}C for 12 hours at which time the oven dry weight per plant was recorded.

On each reading date eight to 16 water agar plates containing fungi producing nematosporangia typical of _P._

graminicolum were isolated in pure culture by means of hyphal tip transfer. All cultures identified had antheridia of monoclinous origin and plerotic oospores, clearly defining them as *P. graminicolum*.

The *P. graminicolum* disease inciting potential was determined in field plots during the 1961 growing season. Crested wheatgrass was planted in crop residue amended field plots at three planting dates. Weekly determinations of percentage *P. graminicolum* recovery from root isolations and mean plant weight were determined as previously described. In addition, plant mortality following the week of maximum emergence was noted.

Fig. 1. Photomicrograph of a wheatgrass root section four days after culturing on 1.5 per cent water agar showing the root section (dark area) and numerous nematosporangia typical of *Pythium graminicolum*



EXPERIMENTAL PROCEDURES AND RESULTS

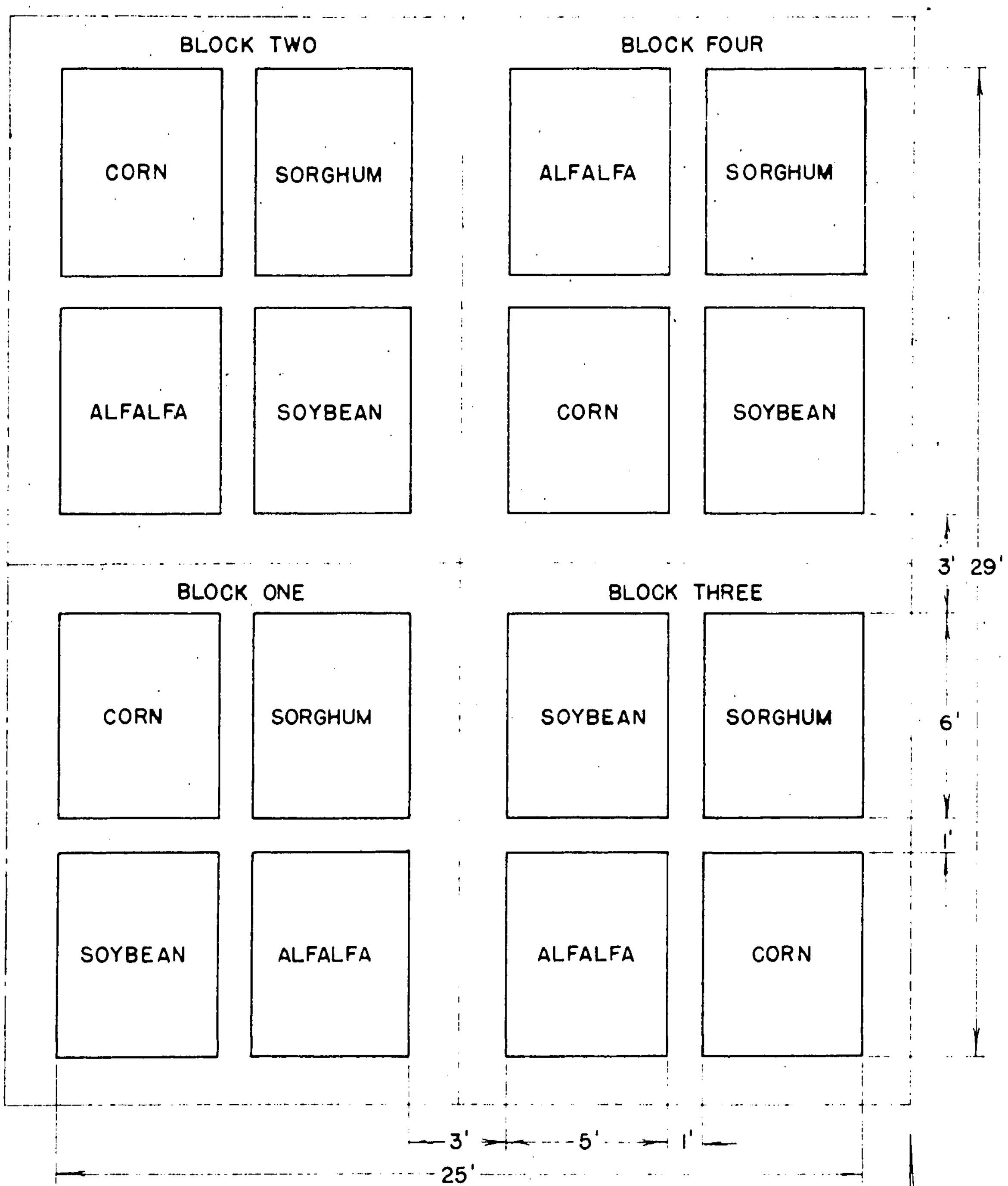
Pythium graminicolum Disease Inciting Potential of Soils Collected Near Roots of Cover Crops

In the spring of 1960 a small field plot (25 X 29 ft.) which had been planted to corn in 1959 was obtained at the Ash Avenue botany and plant pathology field plots in Ames, Iowa. A randomized block design was laid out which consisted of four blocks with each block subdivided into four plots (Fig. 2).

Two legume and two grass cover crops were planted in the four plots within each block on June 4, 1960 (Fig. 2). Single seed lots of soybean, alfalfa, corn and sorghum with laboratory germinations of 92, 90, 96 and 96 per cent respectively were furnished by the Iowa State Seed Testing Laboratory for cover crop seed. Plantings of the above seed were made as follows: soybean, corn and sorghum, three rows two feet apart (Fig. 3); alfalfa, five rows one foot apart (Fig. 4).

Soil collections from the field plots were made at two week intervals on the following dates: June 4, June 18, July 1, July 16, July 13. The first collection was made just prior to sowing the cover crops. Subsequent collections were taken one inch diagonally from the cover crop stems as previously described. Three weeks after planting wheatgrass

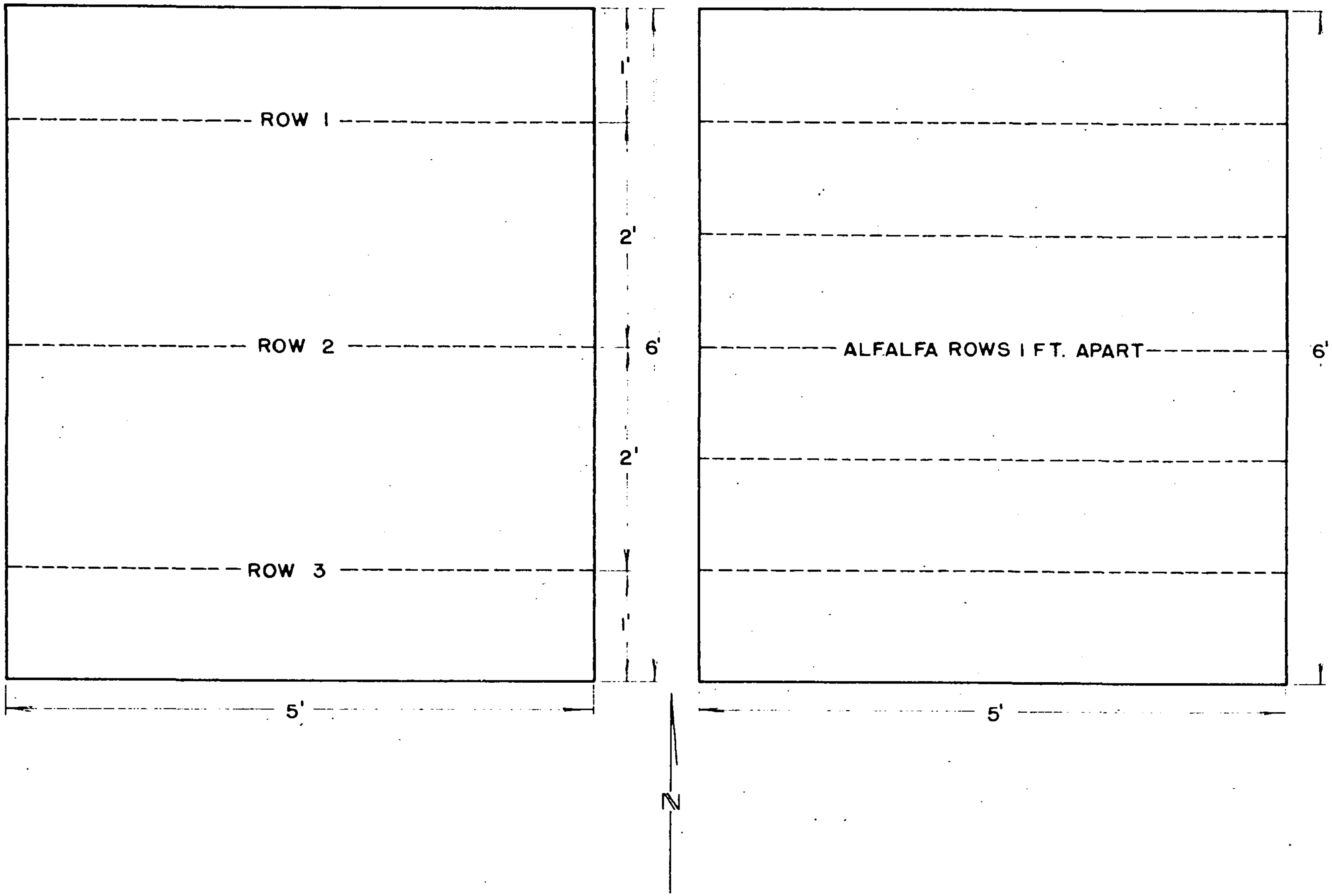
Fig. 2. Diagrammatic representation of the Ash Avenue field plot showing the randomized block design and the four cover crop treatments which were planted within the four plots in each block


BLOCK TWO
CORN
SORGHUM
ALFALFA
SOYBEAN
BLOCK FOUR
ALFALFA
SORGHUM
CORN
SOYBEAN
BLOCK ONE
CORN
SORGHUM
SOYBEAN
ALFALFA
BLOCK THREE
SOYBEAN
SORGHUM
ALFALFA
CORN
3'
29'
6'
1'
3'
5'
1'
25'

SCALE: 1"=5'-0"

Fig. 3. Individual plot planted to soybean, corn or sorghum showing the relative positions of three rows planted June 4, 1960

Fig. 4. Diagrammatic representation of an alfalfa plot showing the relative positions of five rows planted June 4, 1960


1'
ROW 1
2'
ROW 2
6'
2'
ROW 3
1'
5'
ALFALFA ROWS 1 FT. APART
6'
5'
N

seed in the above soil the percentage of surviving plants diseased and percentage recovery of P. graminicolum from root isolations were recorded.

Percentage plants diseased

Average percentages of diseased plants grown in soil collected from near roots of soybean, alfalfa, corn and sorghum at five soil collection dates are graphically illustrated in Fig. 5. Prior to and two weeks after sowing the cover crops (June 4 and June 18 respectively) there were no differences among the percentages of diseased plants grown in the several soil samples. Soil collected from near roots of sorghum plants on July 1, however, produced a relatively large percentage of diseased plants (58 per cent). Soil from soybean plots contained low (35 per cent) and from alfalfa and corn plots intermediate percentages (47 and 50 per cent, respectively) of diseased plants. On July 1 and July 31, soil from sorghum plots maintained a relatively high disease inciting potential as measured by percentages of diseased plants. On the other hand, soybean, alfalfa and corn treated plots steadily decreased in disease inciting potential, with the exception of soybean soil on the July 16 collection date.

An analysis of variance of the data, considering soil collection dates as subplots in a split plot design, was performed (Table 1) with the following results: soils collected from near roots of the two legume and two grass

Fig. 5. Average percentage wheatgrass plants diseased three weeks after planting in soils collected from field plots occupied by roots of four cover crops

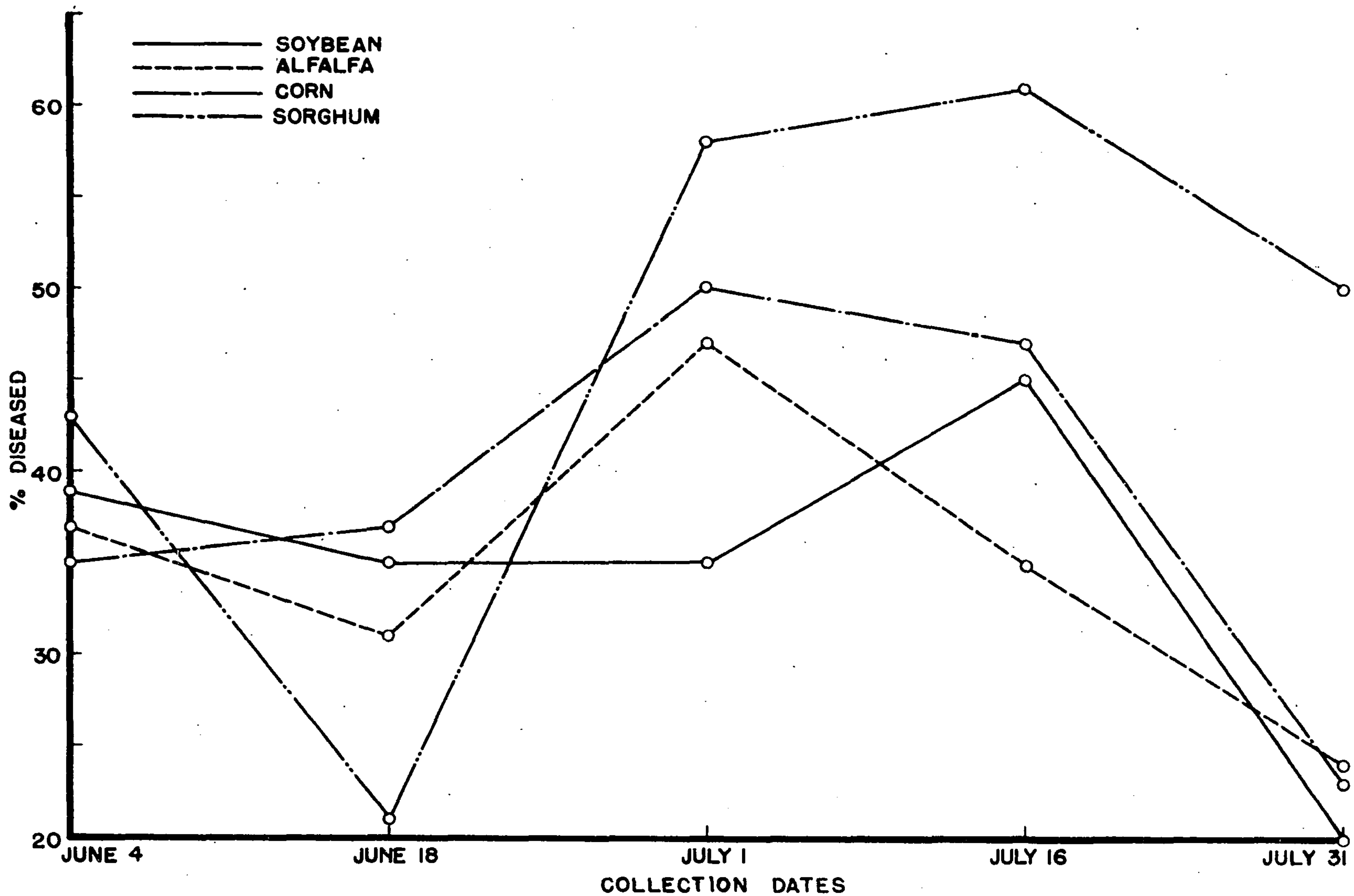

SOYBEAN
ALFALFA
CORN
SORGHUM
% DISEASED
60
50
40
30
20
JUNE 4
JUNE 18
JULY 1
JULY 16
JULY 31
COLLECTION DATES

Table 1. Analysis of variance of diseased wheatgrass plants three weeks after planting in soil collected from near roots of field grown soybean, alfalfa, corn and sorghum cover crops at two week intervals during June and July, 1960

Source of Variation	df	ms	F
Blocks	3	374.9519	
Treatments	3	709.0663	5.095*
Error (a)	9	139.1700	
Dates	4	1533.5138	10.637**
Dates X Treatments	12	301.0711	2.088*
Error (b)	48	144.1660	
Sampling Error	160	97.7374	

*Significant at the .05 probability level.

**Significant at the .01 probability level.

cover crops differed in their disease inciting potential when measured by percentages of diseased plants, as evidenced by the significant F test for "Treatments". Comparisons among the four cover crops over the five collection dates indicate that alfalfa, soybean and corn treated plots did not differ significantly in their effect (Table 2). However, soil from sorghum treated plots produced significantly larger percentages of diseased plants than did soil collected from soybean, alfalfa or corn plots.

Table 2. Average percentage wheatgrass plants diseased when grown in soil collected from near roots of cover crops on five soil collection dates

	Treatments			
	Alfalfa	Soybean	Corn	Sorghum
Average percentage diseased[a]	35.36	35.51	37.61	42.72
Statistical significance*				

[a]Arcsin transformations of percentage data.

*Any two means not underscored by the same line are significantly different at the .05 probability level (Duncan 1955).

Table 3 compares the effect of the four cover crops within each collection date. There were no significant differences among treatments on the June 4 and June 18

Table 3. Average percentage wheatgrass plants diseased when grown in soil collected from near roots of two legume and two grass cover crops on individual collection dates

June 4 soil collection date

	Treatments			
	Alfalfa	Corn	Soybean	Sorghum
Average percentage diseased[a]	36.29	36.74	38.75	40.95
Statistical significance*	———	———	———	———

June 18 soil collection date

	Treatments			
	Sorghum	Alfalfa	Corn	Soybean
Average percentage diseased	26.66	30.29	34.40	35.41
Statistical significance	———	———	———	———

July 1 soil collection date

	Treatments			
	Soybean	Alfalfa	Corn	Sorghum
Average percentage diseased	36.05	43.43	45.10	50.29
Statistical significance	———	———	———	
		———	———	———

[a]Arcsin transformations of percentage data.

*Any two means not underscored by the same line are significantly different at the .05 probability level (Duncan 1955).

Table 3. (Continued)

	July 16 soil collection date			
	Treatments			
	Alfalfa	Soybean	Corn	Sorghum
Average percentage diseased	36.92	40.41	43.52	51.02
Statistical significance	————	————	————	
			————	————

	July 31 soil collection date			
	Treatments			
	Soybean	Corn	Alfalfa	Sorghum
Average percentage diseased	26.86	28.43	29.85	44.68
Statistical significance	————	————	————	————

collection dates, but on July 1 the disease inciting potential of soil from the sorghum plots was significantly larger than soybean; on July 16, larger than both alfalfa and soybean; on July 31, significantly greater than soybean, corn and alfalfa. No significant differences among soybean, corn and alfalfa treatments occurred on any collection date.

Mean top height of the cover crops and average pH of each soil sample were recorded at the time of the June 18 through July 31 collection dates. There were no consistent

Table 4. Average cover crop top height and pH of soil samples collected from near roots of two legume and two grass cover crops on four collection dates

Soil collection dates	COVER CROP SOYBEAN top ht. (inches)	SOYBEAN soil pH	ALFALFA top ht. (inches)	ALFALFA soil pH	CORN top ht. (inches)	CORN soil pH	SORGHUM top ht. (inches)	SORGHUM soil pH
June 18	3.5[a]	6.4[b]	1.4	6.3	7.0	6.4	3.5	6.3
July 1	7.2	6.2	4.2	6.1	17.0	6.1	14.7	6.1
July 16	14.6	6.1	6.6	6.1	31.7	6.2	22.4	6.2
July 31	29.1	6.3	7.4	6.2	55.2	6.4	42.0	6.1

[a]Each number is the average of 12 readings (three readings per plot, one plot in each of four blocks).

[b]Each number is the average of 20 readings (five readings per sample, four samples per treatment).

differences in soil pH associated with any of the four cover crops (Table 4).

Percentage recovery of *Pythium graminicolum*

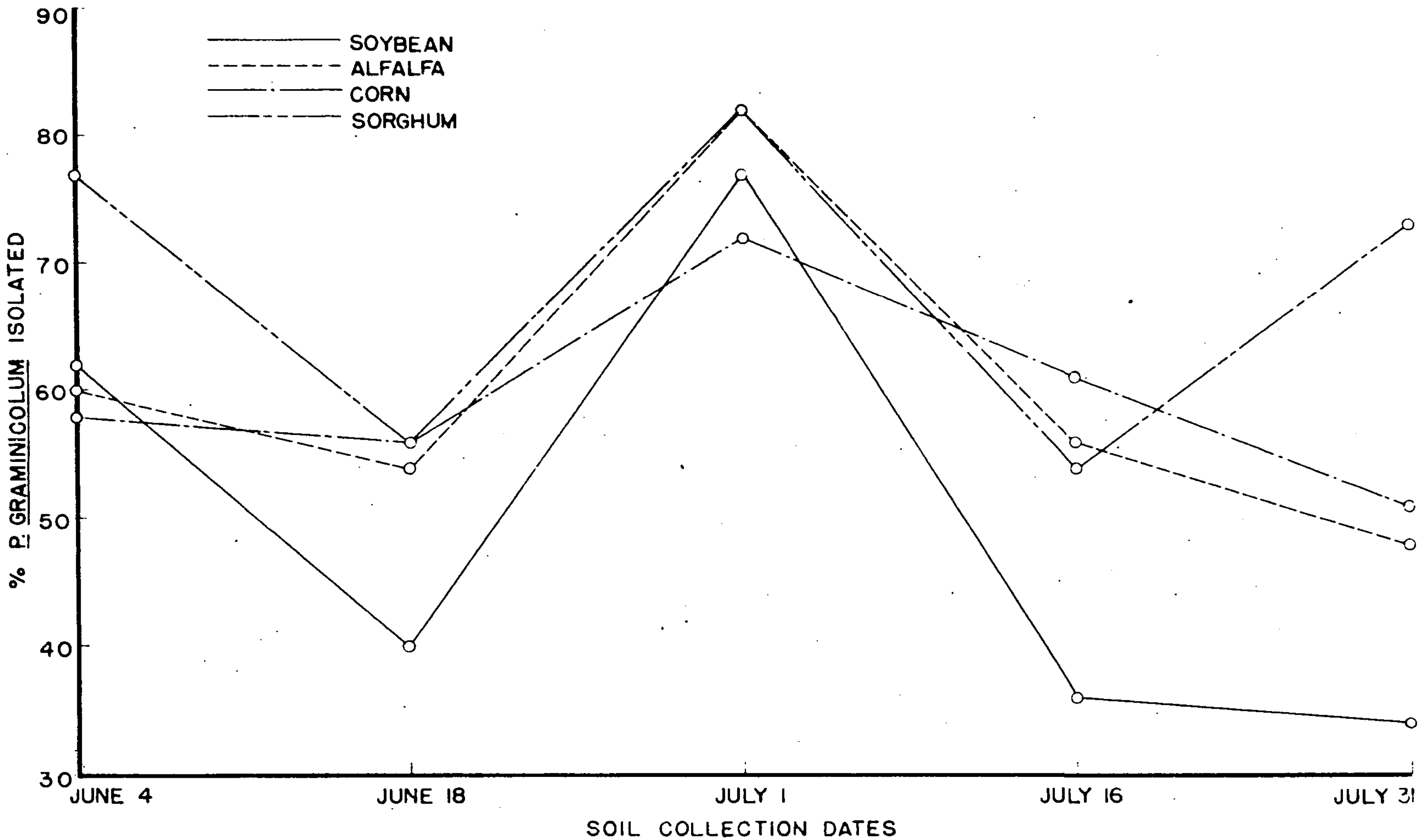
Average percentage recovery of *P. graminicolum* from roots of wheatgrass plants grown in soil collected from field plots occupied by roots of soybean, alfalfa, corn and sorghum at five soil collection dates is depicted graphically in Fig. 6. Total numbers of cultures examined for each treatment on the five dates are shown in Table 5.

Table 5. Total numbers of root isolations from wheatgrass plants grown in soil collected at two week intervals from plots supporting two legume and two grass cover crops

Soil collection date	Cover Crops			
	Soybean	Alfalfa	Corn	Sorghum
June 4	108	108	108	108
June 18	144	144	144	144
July 1	150	144	144	145
July 16	144	144	144	142
July 31	142	144	144	144

Recovery of *P. graminicolum* from roots of wheatgrass plants grown in soil samples from the soybean, alfalfa and corn plots prior to sowing the cover crops (June 4) was about equal, varying from 58 per cent for corn to 62 per cent

Fig. 6. Average percentage recovery of *Pythium graminicolum* from root isolations of wheatgrass plants grown in soil collected from field plots supporting two legume and two grass cover crops


SOYBEAN
ALFALFA
CORN
SORGHUM
% P. GRAMINICOLUM ISOLATED
90
80
70
60
50
40
30
JUNE 4
JUNE 18
JULY 1
JULY 16
JULY 31
SOIL COLLECTION DATES

for soybean (Fig. 6). However, isolations from roots grown in soil samples collected from sorghum plots produced 77 per cent recovery as compared to 58 per cent for corn.

Isolations from plants grown in soil samples taken June 18 and July 1 showed little difference in recovery data among treatments except for the 16 percentage point discrepance between soybean (40 per cent) and corn and sorghum (56 per cent) on the June 18 collection date.

Isolations from plants grown in soybean soil samples collected July 16 demonstrated a marked decrease in percentage _P. graminicolum_ recovery as compared to recovery from roots of plants grown in soil samples taken from near roots of the other three crops. On July 31, percentage _P. graminicolum_ recovery from plants grown in soil from soybean plots was again low (34 per cent), whereas isolations from roots of wheatgrass grown in soil from sorghum plots yielded a much larger percentage of this fungus (73 per cent). Isolations from wheatgrass plants grown in soil collected July 31 from alfalfa and corn plots yielded intermediate percentages of _P. graminicolum_ as compared to recovery data from plants sown in soil from sorghum and soybean plots.

Analysis of variance of arcsin transformations of percentage _P. graminicolum_ recovery data considering soil collection dates as sub-plots in a split-plot design is shown in Table 6. There were no statistical differences among treatments with respect to percentage _P. graminicolum_

Table 6. Analysis of variance of percentage recovery of *Pythium graminicolum* from roots of wheatgrass plants grown in soil collected from near roots of two legume and two grass cover crops at two week intervals during June and July, 1960

Source of Variation	df	ms	F
Blocks	3	588.0796	
Treatments	3	702.1360	3.48
Error (a)	9	201.4911	
Dates	4	944.7641	5.09**
Dates X Treatments	12	108.7444	0.59
Error (b)	48	185.5381	

**Significant at the .01 probability level.

recovery.

Cover Crop Incorporation

During the first week in November, tops of the two legume and two grass cover crops were incorporated in the soil of the plots in which they were grown as follows: corn plants were cut at the soil line, stripped of leaves, tassels and ears, and the remaining stems chopped into small pieces. Corn stem residue was replaced on each corn plot at a rate of 14 lbs. residue per 5 X 6 ft. plot, which was equivalent to 10 tons per acre. Each corn plot was spaded and worked with a hoe to incorporate the corn stover in the top six inches of soil. Sorghum and soybean plots were treated in much the same manner in that only chopped stem residue, at the rate of 14 lbs. per plot, was incorporated in the respective plots. Each alfalfa plot received 2.8 lbs. per plot of stem and leaf residue which was equivalent to about two tons per acre. All plots were thoroughly worked to obtain as homogenous a soil-residue mixture as possible. Plots thus treated were used for subsequent tests the following growing season.

Effect of Two Legume and Two Grass Crop Residues on *Pythium graminicolum* Disease Inciting Potential

In mid-April of 1961 the soil in the residue amended field plots was sufficiently dry to be worked with spade and hoe. Care was taken to remove soil particles from the tools between plots to prevent mixing soil treatments. In addition, wooden frames four inches high were built around each of the 16 plots to prevent contamination by surface washing of soil.

Disease inciting potential determined in the greenhouse

Percentage plants diseased   Fig. 7 graphically illustrates the average percentages of diseased wheatgrass plants produced when grown in soil samples collected April 25 and May 25 from residue amended field plots. The four crop residue treatments resulted in similar disease inciting potentials on April 25 (Table 7). On May 25, however, the percentage of diseased plants in soil from sorghum treated plots was significantly larger than in soil from the soybean, alfalfa and corn amended plots (Tables 8 and 9). In addition, soil from soybean treated plots produced significantly smaller percentages of diseased plants than soil from those plots amended with corn and sorghum (Table 9).

Percentage recovery of *Pythium graminicolum*   Percentage recovery of *P. graminicolum* from root isolations of wheatgrass plants grown in soil samples collected April and May 25 from residue amended field plots is graphically depicted in Fig. 8. Plants grown in soybean amended soil produced the smallest, sorghum the largest, and alfalfa and corn intermediate percentages of cultures yielding *P. graminicolum* at both soil collection dates. Comparisons between the two

Fig. 7. Average percentage wheatgrass plants diseased four weeks after planting in soil samples collected April 25 and May 25 from two legume and two grass crop residue amended field plots

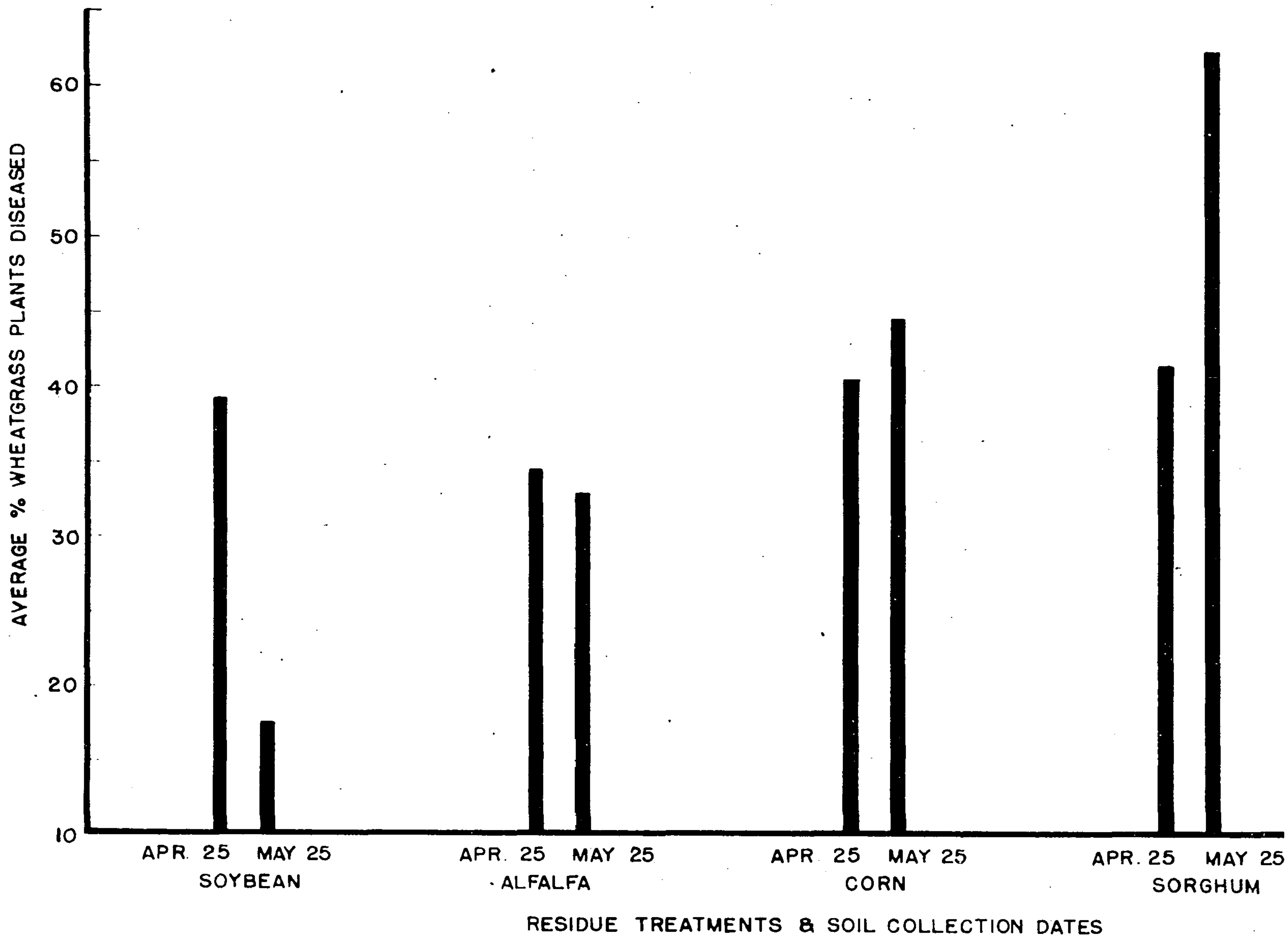

AVERAGE % WHEATGRASS PLANTS DISEASED
60
50
40
30
20
10
APR. 25
MAY 25
SOYBEAN
APR. 25
MAY 25
ALFALFA
APR. 25
MAY 25
CORN
APR. 25
MAY 25
SORGHUM
RESIDUE TREATMENTS & SOIL COLLECTION DATES

Table 7. Analysis of variance of percentage plants diseased four weeks after planting in soil collected April 25 from soybean, alfalfa, corn and sorghum amended plots

Source of Variation	df	ms	F
Blocks	3	1497.0913	
Treatments	3	231.6866	1.18
Experimental Error	9	195.6528	
Sampling Error	48	257.0536	

Table 8. Analysis of variance of percentage plants diseased four weeks after planting in soil collected May 25 from soybean, alfalfa, corn and sorghum amended plots

Source of Variation	df	ms	F
Blocks	3	677.0719	
Treatments	3	1830.6978	6.29*
Experimental Error	9	290.8248	
Sampling Error	32	181.7877	

*Significant at the .05 probability level.

Fig. 8. Average percentage recovery of *Pythium graminicolum* from wheatgrass plants four weeks after planting in soil samples collected April 25 and May 25 from two legume and two grass crop residue amended field plots

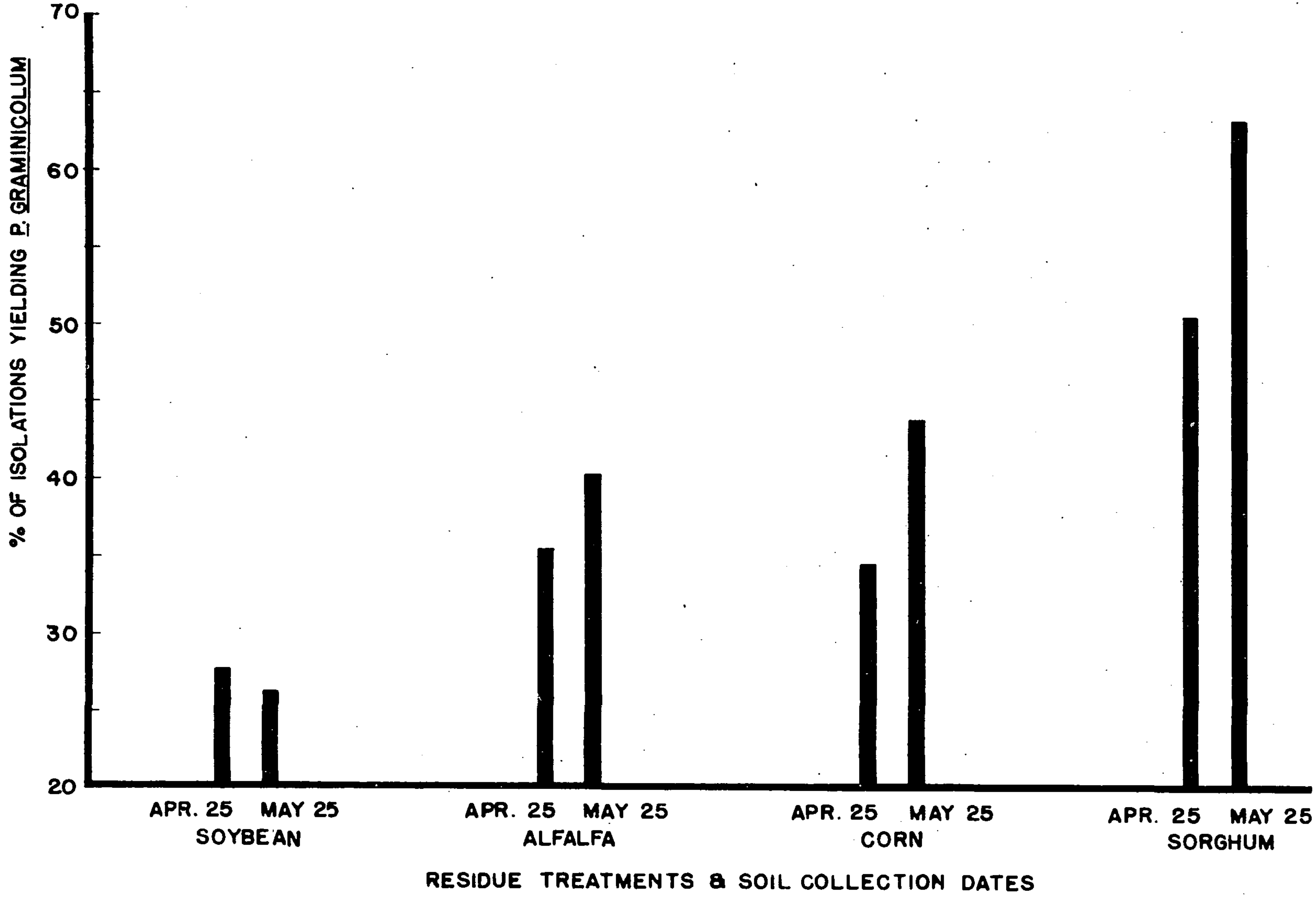

70
60
50
40
30
20
% OF ISOLATIONS YIELDING P. GRAMINICOLUM
APR. 25 MAY 25
SOYBEAN
APR. 25 MAY 25
ALFALFA
APR. 25 MAY 25
CORN
APR. 25 MAY 25
SORGHUM
RESIDUE TREATMENTS & SOIL COLLECTION DATES

Table 9. Average percentage diseased wheatgrass plants grown in soil collected May 25 from soybean, alfalfa, corn and sorghum amended field plots

	Treatments			
	Soybean	Alfalfa	Corn	Sorghum
Average percentage diseased[a]	22.85	33.80	39.39	52.54
Statistical significance*	———	——— ———	———	———

[a]Arcsin transformations of percentage data.

*Any two means not underscored by the same line are significantly different at the .05 probability level (Duncan 1955).

collection dates show an increase in percentage recovery on May 25 for each treatment except soybean, for which a slight decrease was recorded.

Percentage recovery analysis of variance and statistical comparisons among treatments on the two collection dates are shown in Tables 10 through 13. Table 10 shows that isolations from roots of wheatgrass plants grown in soil samples collected April 25 yielded statistically different percentages of _P. graminicolum_. Comparisons among treatments (Table 11) indicate that only the soybean and sorghum treated soils differed with respect to their _P. graminicolum_ disease inciting potentials as measured by percentage recovery data.

Percentage recovery analysis of variance from wheatgrass

Table 10. Analysis of variance of percentage recovery of *P. graminicolum* from wheatgrass plants four weeks after planting in soil samples collected April 25 from field plots amended with two legume and two grass crop residues

Source of Variation	df	ms	F
Blocks	3	364.8958	
Treatments	3	234.2557	3.89*
Experimental Error	9	60.1611	

*Significant at the .05 probability level.

Table 11. Average percentage recovery of *P. graminicolum* from roots of wheatgrass plants grown in soil samples collected April 25 from field plots amended with two legume and two grass crop residues

	Treatments			
	Soybean	Alfalfa	Corn	Sorghum
Average percentage recovery[a]	25.65	35.76	35.85	45.39
Statistical significance*				

[a]Arcsin transformations of percentage data.

*Any two means not underscored by the same line are significant at the .05 probability level (Duncan 1955).

plants grown in soil samples collected May 25 is shown in Table 12. The residue treatments affected the P. graminicolum disease inciting potential as indicated by the significant F test for "Treatments", and a statistical comparison among treatments shows that the percentage recovery from plants grown in sorghum treated soil was significantly greater than in soil from soybean, alfalfa or corn treated plots (Table 13).

Disease inciting potential determined in the field

The effect of two legume and two grass crop residues on the P. graminicolum disease inciting potential was tested in the field as follows: the 16 residue amended field plots were divided into three two-foot sections in which crested wheatgrass was planted on three planting dates (Fig. 9). The first planting date (April 30) 100 wheatgrass seed units were planted in each of 10 rows in section one (Fig. 9). The two outermost rows were considered as border rows so were not used in later tests. The middle rows were numbered one through eight and subsequently used to elucidate differences in P. graminicolum disease inciting potentials of the residue amended plots. The above planting procedures were repeated May 15 and June 5 in sections two and three respectively.

Weekly readings of seedling emergence and plant survival were made until the plants, in two row groups, were "terminated" as follows: four weeks after sowing, plants in rows one and two were removed and the root systems washed free of

Table 12. Analysis of variance of percentage recovery of *P. graminicolum* from wheatgrass plants four weeks after planting in soil samples collected May 25 from field plots amended with two legume and two grass crop residues

Source of Variation	df	ms	F
Blocks	3	82.2799	
Treatments	3	813.6353	5.45*
Error	9	149.4138	

*Significant at the .05 probability level.

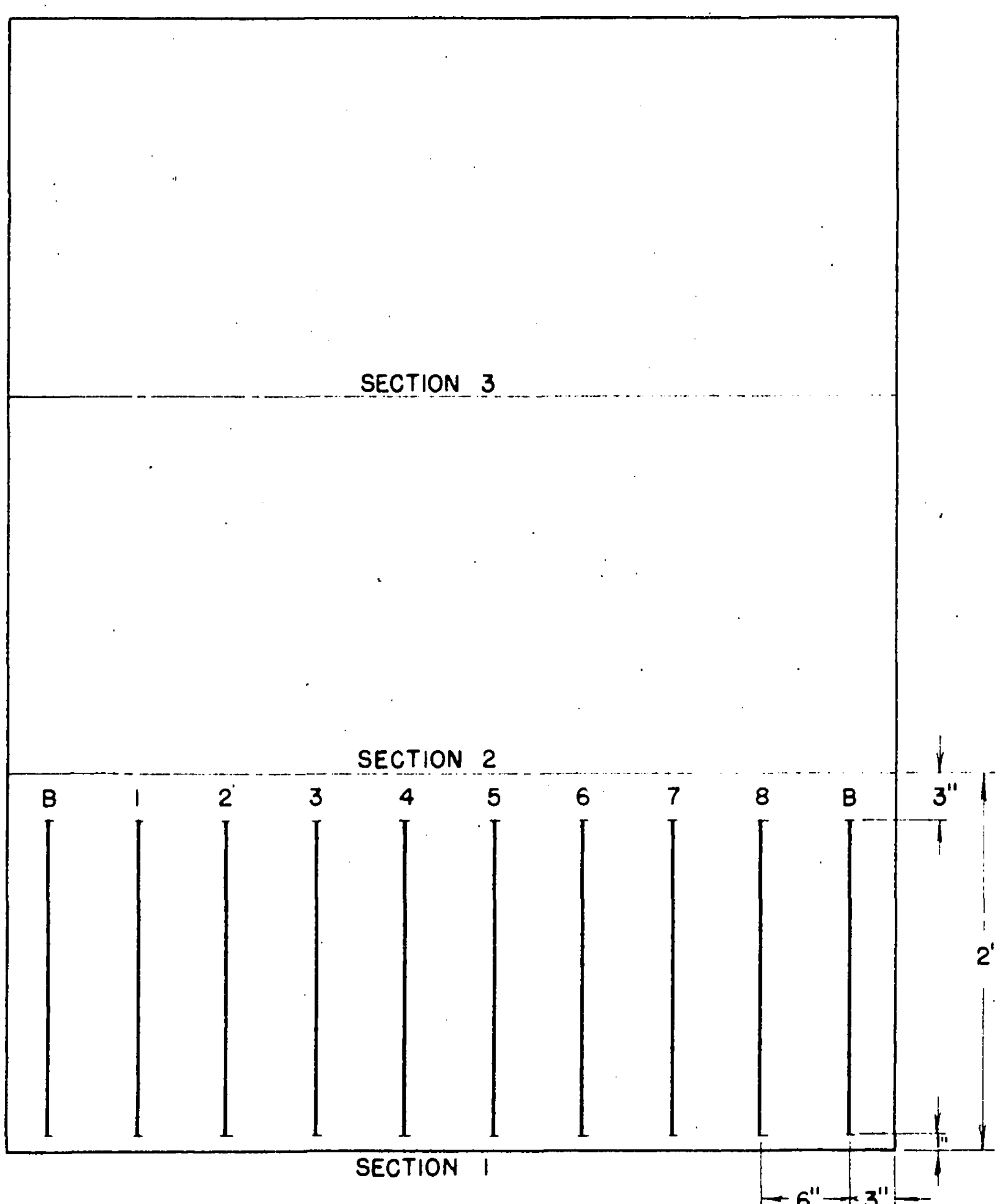
Table 13. Average percentage recovery of *P. graminicolum* from roots of wheatgrass plants grown in soil samples collected May 25 from field plots amended with two legume and two grass crop residues

	Treatments			
	Soybean	Alfalfa	Corn	Sorghum
Average percentage recovery[a]	35.99	47.15	50.00	70.21
Statistical significance*				

[a]Arcsin transformations of percentage data.

*Any two means not underscored by the same line are significant at the .05 probability level (Duncan 1955).

Fig. 9. Diagrammatic representation of a field plot previously amended with soybean, alfalfa, corn or sorghum residue. Each plot was divided into three sections representing three crested wheatgrass planting dates as follows: section one, April 30; section two, May 15; section three, June 5.



B = BORDER ROW
1-8 = NUMBER OF ROW
100 SEED UNITS PER ROW

adhering soil particles. Root isolations and oven dry weight determinations were made as previously described. The above procedure was repeated at weekly intervals until all eight rows in each section had been terminated.

Plant mortality In sections one and two (April 30 and May 15 planting dates) the maximum numbers of wheatgrass plants emerged the third week after planting (Tables 14 and 15), so mortality readings were made one through four weeks following the maximum emergence dates.

As shown in Table 14, the largest wheatgrass plant mortality was in sorghum amended soils, whereas the least numbers died in soybean plots and intermediate numbers succumbed when planted in alfalfa and corn plots. There was an increase in numbers of plants that died for all treatments at every reading date in section two (May 15 planting date); however, the same general relationship reoccurred with largest mortality in sorghum, least in soybean, and intermediate numbers in alfalfa and corn amended plots (Table 15).

In section 3 (June 5 planting date) maximum numbers of wheatgrass plants emerged two weeks after planting, and mortality readings were taken one through five weeks following the maximum emergence date. In general, mortality in soybean amended plots was lowest, in sorghum plots the highest, and in corn and alfalfa plots intermediate one and two weeks after maximum plant emergence (Table 16). On subsequent reading

Table 14. Two row totals of wheatgrass plant mortality at weekly inter
field plots previously amended with two legume and grass cro

Planting date 1 (April 30)

		RES			
	Weeks after	SOYBEAN wheatgrass plants		ALFALFA wheatgrass plants	
Row number	maximum emergence	e- merged	number died	e- merged	numbe died
1 and 2		260[a]		266	
	one[b]		8[c]		21
3 and 4		275		254	
	one		14		28
	two[b]		19		44
5 and 6		236		241	
	one		16		23
	two		16		31
	three[b]		25		31
7 and 8		251		216	
	one		12		14
	two		21		19
	three		21		22
	four[b]		26		28

[a]Each number is the maximum number of plants produced three weeks a
four plots previously amended with the indicated crop residue.

[b]The surviving plants in the indicated rows were removed from the s
determinations.

[c]Each number is the total numbers of plants that died in the indica
indicated crop residue.

at weekly intervals following the week of maximum emergence in
e and grass crop residues

RESIDUE TREATMENTS					
ALFALFA		CORN		SORGHUM	
eatgrass plants		wheatgrass plants		wheatgrass plants	
ed	number died	e-merged	number died	e-merged	number died
		271		235	
	21		30		53
		256		245	
	28		41		41
	44		44		53
		239		242	
	23		29		33
	31		30		49
	31		44		50
		234		257	
	14		26		33
	19		28		42
	22		31		49
	28		37		50

ed three weeks after planting 200 wheatgrass seed in each of the
sidue.

moved from the soil for subsequent isolations and dry weight

ed in the indicated rows in four plots amended with the

Table 15. Two row totals of wheatgrass plant mortality at weekly in field plots previously amended with two legume and grass

Planting date 2 (May 15)					
		SOYBEAN		ALFALFA	
	Weeks after	wheatgrass plants		wheatgrass pla	
Row number	maximum emergence	e-merged	number died	e-merged	nu di
1 and 2		259[a]		341	
	one[b]		18[c]		5
3 and 4		274		303	
	one		13		5
	two[b]		36		12
5 and 6		290		378	
	one		18		2
	two		42		6
	three[b]		63		7
7 and 8		306		297	
	one		20		5
	two		51		10
	three		59		10
	four[b]		79		11

[a]Each number is the maximum number of plants produced three weel plots previously treated with the indicated crop residue.

[b]The surviving plants in the indicated rows were removed from th determinations.

[c]Each number is the total numbers of plants that died in the inc indicated crop residue.

ty at weekly intervals following the week of maximum emergence in
gume and grass crop residues

RESIDUE TREATMENTS					
ALFALFA		CORN		SORGHUM	
wheatgrass plants		wheatgrass plants		wheatgrass plants	
- erged	number died	e- merged	number died	e- merged	number died
341		310		233	
	57		62		71
303		287		293	
	55		58		71
	128		92		109
378		276		336	
	28		38		58
	68		74		84
	77		84		92
297		266		316	
	52		37		59
	103		74		116
	108		80		124
	115		91		134

luced three weeks after planting 200 wheatgrass seed in each of four
lue.

removed from the soil for subsequent isolations and dry weight

died in the indicated rows in four plots amended with the

Table 16. Two row totals of wheatgrass plant mortality at weekly inter field plots previously amended with two legume and grass crc

Planting date 3 (June 5)		RES			
	Weeks after maximum emergence	SOYBEAN wheatgrass plants		ALFALFA wheatgrass plants	
Row number		e-merged	number died	e-merged	numbe died
1 and 2		216[a]		218	
	one		25[b]		36
	two[c]		37		59
3 and 4		240		291	
	one		28		64
	two		43		75
	three[c]		60		87
5 and 6		275		196	
	one		39		26
	two		54		28
	three		66		38
	four[c]		71		47
7 and 8		229		225	
	one		34		47
	two		76		61
	three		80		73
	four		87		78
	five[c]		106		95

[a]Each number is the maximum number of plants produced two weeks aft plots previously treated with the indicated crop residue.

[b]Each number is the total numbers of plants that died in the indica indicated crop residue.

[c]The surviving plants in the indicated rows were removed from the s determinations.

ty at weekly intervals following the week of maximum emergence in
gume and grass crop residues

RESIDUE TREATMENTS					
ALFALFA		CORN		SORGHUM	
wheatgrass plants		wheatgrass plants		wheatgrass plants	
rged	number died	e-merged	number died	e-merged	number died
18		263		208	
	36		41		41
	59		53		57
91		261		290	
	64		47		33
	75		66		45
	87		86		59
96		243		270	
	26		45		45
	28		55		50
	38		59		52
	47		88		62
25		229		241	
	47		47		55
	61		72		57
	73		76		65
	78		78		72
	95		79		93

uced two weeks after planting 100 wheatgrass seed in each of four
ue.

lied in the indicated rows in four plots amended with the

removed from the soil for subsequent isolations and dry weight dates no residue treatment differences were evident with respect to plant mortality.

<u>Percentage recovery of *Pythium graminicolum*</u> Four, five, six and seven weeks after sowing crested wheatgrass seed in the three sections of each residue amended field plot, two row units of plants were removed from the plots in which they were grown. Plants removed from each plot were treated as follows: 36 root sections from 12 plants, chosen at random, were placed in petri plates containing about 15 ml of 1.5 per cent water agar. As each residue treatment was replicated four times, there were 144 root isolations per treatment at each isolation date. Average percentages of the above cultures yielding *P. graminicolum* are shown in Table 17. *P. graminicolum* was most often recovered from plants grown in sorghum treated plots at each planting date. Isolations from roots of plants grown in soybean, alfalfa and corn plots yielded inconsistent percentages of *P. graminicolum* with no one treatment consistently affecting recovery data.

<u>Mean plant weights</u> Plants removed from residue amended field plots and not used in isolation procedures were dried for 12 hours at 100 ^{o}C. Table 18 shows the average oven dry weight per plant produced four, five, six and seven weeks after planting at the three planting dates. Soybean amended plots produced the heaviest plants, sorghum the lightest, and corn and alfalfa intermediate weight plants

Table 17. Percentage recovery of *Pythium graminicolum* from roots of wheatgrass plants four, five, six and seven weeks after planting, at three planting dates, in field plots previously amended with two legume and two grass crop residues

Weeks after planting	RESIDUE TREATMENTS SOYBEAN percentage recovery	ALFALFA percentage recovery	CORN percentage recovery	SORGHUM percentage recovery
		Planting date 1 (April 30)		
four	14.6[a]	12.5	24.3	49.3
five	29.9	30.6	45.1	54.9
six	60.0	54.9	55.6	66.7
seven	45.0	26.4	33.3	54.9
		Planting date 2 (May 15)		
four	25.0	43.1	33.3	58.3
five	34.0	30.6	25.7	40.3
six	41.7	21.6	28.5	54.2
seven	52.1	9.7	6.3	23.6
		Planting date 3 (June 5)		
four	27.1	16.0	34.7	50.0
five	36.8	45.1	42.4	68.1
six	71.5	58.3	41.0	71.5
seven	31.3	29.2	27.1	35.4

[a]Each number is the average percentage recovery from 144 root isolations (four replicates, 36 isolations per replicate).

Table 18. Average oven dry weights per wheatgrass plant weighed four, five, six and seven weeks after planting in field plots previously amended with two legume and two grass crop residues

Weeks after planting	RESIDUE TREATMENTS SOYBEAN ave. dry weight, mg	ALFALFA ave. dry weight, mg	CORN ave. dry weight, mg	SORGHUM ave. dry weight, mg
		Planting date 1 (April 30)		
four	9.9[a]	7.3	7.9	8.7
five	34.9	21.9	20.0	17.5
six	50.2	38.0	38.0	19.8
seven	76.2	53.6	59.0	21.3
		Planting date 2 (May 15)		
four	8.7	7.2	7.5	5.3
five	14.0	10.9	11.2	11.5
six	36.4	32.3	31.8	13.3
seven	62.9	44.3	46.9	20.9
		Planting date 3 (June 5)		
four	17.1	17.5	20.0	15.3
five	38.8	26.9	24.6	28.1
six	68.6	76.3	40.5	43.6
seven	74.1	57.1	51.0	43.2

[a]Each number is the average oven dry weight per plant produced in two rows within four residue amended field plots.

for the April 30 and May 15 planting dates. On the June 5 planting date no residue treatment consistently affected mean plant weight.

Effect of Soybean Green Manure on *Pythium graminicolum* Disease Inciting Potential

By June 23 the eight rows of wheatgrass planted in section one of the 16 field plots had been terminated (Figs. 2 and 9). Section one in blocks one and two was planted to soybeans, using the same seed as in the 1960 cover crop experiment. By July 19 the third trifoliate leaf of the majority of soybean plants had fully expanded, and at this time the plants were incorporated into the soil as follows: plants growing in block one were cut at the soil line and 4.6 lbs. (equivalent to 10 tons per acre) of the tops were chopped up and incorporated in each 2 X 5 foot section; therefore, both tops and roots were incorporated in the plots in this block. Soybean tops removed from block two were chopped up and incorporated in section one of the plots in block three at the rate of 10 tons fresh weight per acre. Section one of the plots in block four received 224 grams of purified potato starch powder and 13 grams of urea which was roughly equivalent to the amount of carbon and nitrogen present in 10 tons per acre of soybean tops. Plots thus treated were thoroughly hand worked to obtain as homogenous a soil-residue mixture as possible.

Soil samples from the above plots were taken to the greenhouse and assayed for their _P. graminicolum_ disease inciting potential. The first soil collection was taken one week following the green manure incorporations; subsequent collections were made at weekly intervals for five weeks. At each collection date soil samples were taken from section two of the plots in block four (Figs. 2 and 9) which received no green manurial treatment. Soil samples thus collected were considered as nontreated checks.

Green manure effects in sorghum plots

In previous tests field plots planted to and amended with sorghum consistently demonstrated the largest _P. graminicolum_ disease inciting potentials. The effects of soybean tops, tops plus roots, roots, and starch plus urea amendments incorporated in the sorghum plots on the percentages of wheatgrass plants diseased and mean plant weights are depicted graphically in Figs. 10 and 11. As compared to the nontreated checks, the above soil amendments demonstrated the following: soybean tops and tops plus roots treatments effectively reduced the percentage of diseased wheatgrass plants grown in soil samples collected one, two and three weeks following the residue incorporation (Fig. 10). A corresponding increase in mean wheatgrass plant weights occurred for a period of three weeks following the tops and tops plus roots treatments (Fig. 11). Plants grown in starch plus urea amended plots consistently yielded

Fig. 10. Average percentages of wheatgrass plants diseased four weeks after planting in soil samples collected from field plots one, two, three, four and five weeks after incorporation of green soybean tops, tops plus roots, roots, and starch plus urea soil amendments

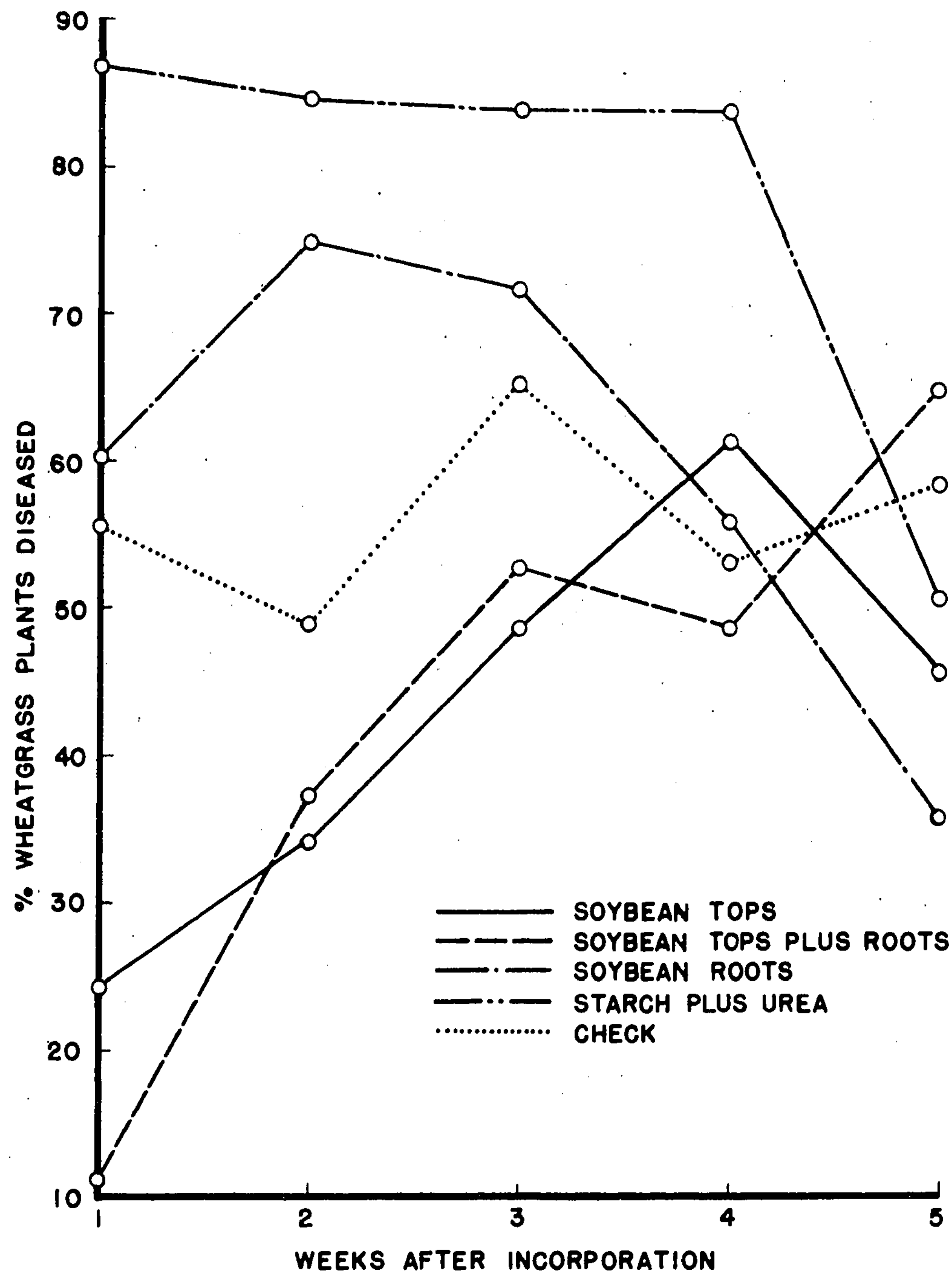
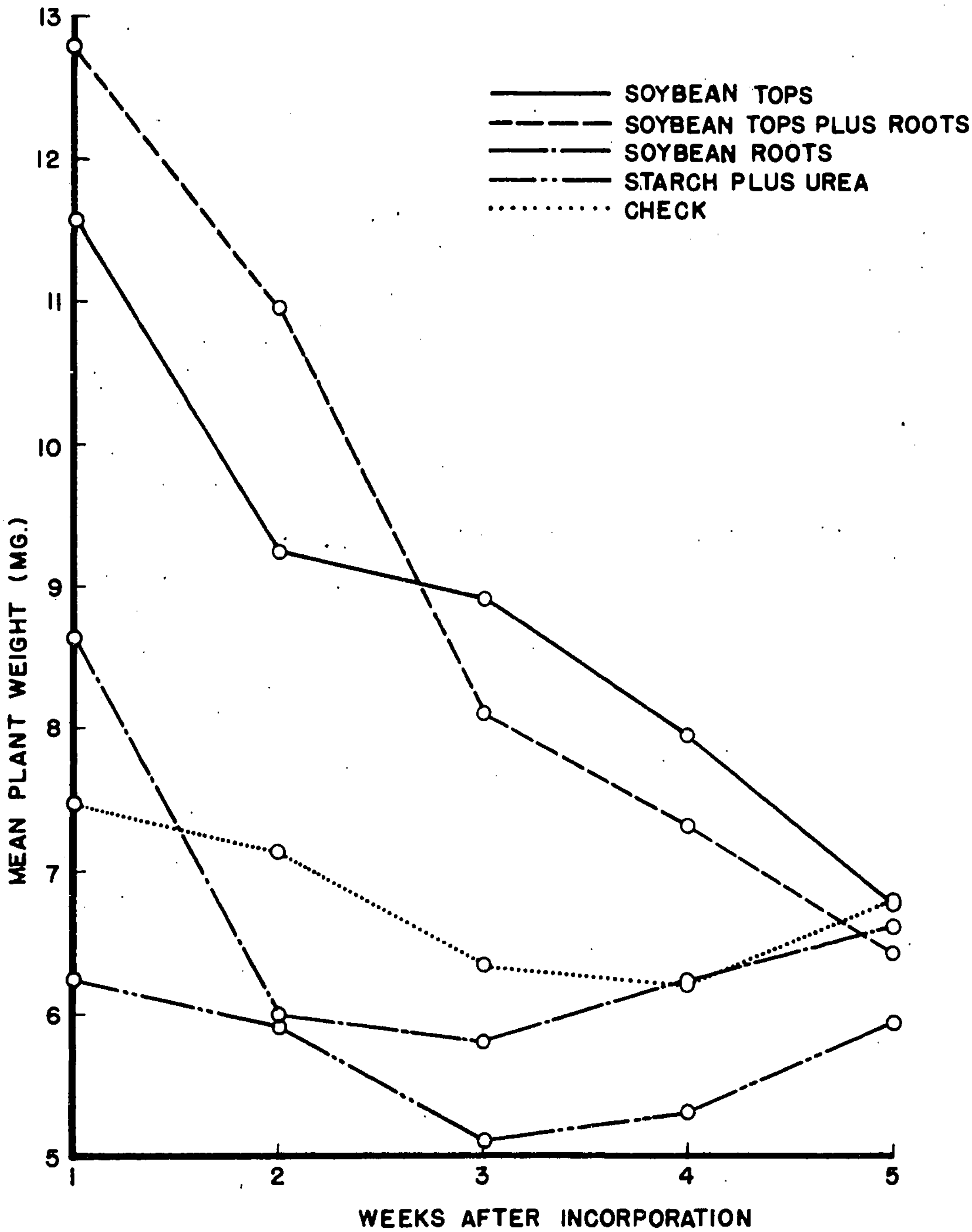

90
80
70
60
50
40
30
20
10
% WHEATGRASS PLANTS DISEASED
1
2
3
4
5
WEEKS AFTER INCORPORATION
SOYBEAN TOPS
SOYBEAN TOPS PLUS ROOTS
SOYBEAN ROOTS
STARCH PLUS UREA
CHECK

Fig. 11. Mean plant weight, in milligrams, four weeks after sowing crested wheatgrass seed in soil samples collected from field plots one, two, three, four and five weeks after incorporation of green soybean tops, tops plus roots, roots, and starch plus urea soil amendments


SOYBEAN TOPS
SOYBEAN TOPS PLUS ROOTS
SOYBEAN ROOTS
STARCH PLUS UREA
CHECK
MEAN PLANT WEIGHT (MG.)
13
12
11
10
9
8
7
6
5
1
2
3
4
5
WEEKS AFTER INCORPORATION

the lightest plants regardless of the soil collection date; however, no consistent effect of soybean root amendments on mean wheatgrass plant weights was apparent (Fig. 11).

Green manure effects in soybean, alfalfa, corn and sorghum plots

Table 19 shows the average percentage wheatgrass plants diseased and mean oven dry weights per plant four weeks after sowing seed in soils collected from soybean green manure amended plots which were previously planted to and amended with mature soybean, alfalfa, corn and sorghum. The largest percentages of diseased plants in the check soil occurred when wheatgrass was sown in soil samples collected from the plot previously amended with sorghum residue. Intermediate numbers of plants were diseased in the plots formerly amended with corn and alfalfa residues, and the least numbers of diseased plants occurred in soil from the soybean plot. The same relationship occurred with respect to the mean plant weights in that the heaviest plants were produced in soil samples from the soybean plot, intermediate weights in alfalfa and corn, and the lightest plants in soil from the sorghum plot. The above relationships were apparent at each of the five soil collections.

When the green manurial treatment included soybean tops (tops and tops plus roots amendments) there tended to be a decrease in percentage of diseased plants and an increase in mean plant weight. The above "top" effect was most apparent in those plots previously showing a large disease inciting potential (sorghum plots).

In comparison with the nontreated check, soybean root amendments tended to increase incidence of disease, regardless of the previous soil treatment. This effect was especially apparent in soil collections taken one and two weeks after the root incorporation.

Starch plus urea amendments had no effect on the numbers of diseased wheatgrass plants grown in soil samples from plots previously amended with soybean, alfalfa or corn residues. There was a large increase in mean wheatgrass plant weight when grown in soil from the plot previously amended with soybean residue, but only in the collection taken one week following the starch plus urea amendment.

Table 19. Average percentage of plants diseased and mean plant weights four samples collected at weekly intervals for five weeks from field p alfalfa, corn and sorghum and subsequently amended with green soy starch plus urea

Previous crop incorpo- ration	Soybean soil amendment	WEEKS AFTER INCO ONE dis.[a] %	mean wt.[b] mg	TWO dis. %	mean wt. mg	THR dis. %	m
soybean		8.0	15.7	12.8	12.7	17.5	
alfalfa		17.3	13.0	25.6	10.9	24.9	
corn	tops	10.0	12.8	19.0	10.4	14.5	
sorghum		24.4	11.6	34.3	9.3	48.7	
soybean		19.9	12.4	8.4	11.0	19.4	
alfalfa	tops	15.9	12.8	15.7	12.0	25.2	
corn	plus	4.5	12.4	32.4	10.9	28.1	
sorghum	roots	11.2	12.8	37.4	11.0	52.8	
soybean		31.9	12.5	27.8	10.0	35.6	
alfalfa		63.8	8.9	69.3	6.5	34.5	
corn	roots	33.3	9.7	39.3	10.7	54.4	
sorghum		60.3	8.6	75.0	6.0	71.6	
soybean		21.0	16.3	21.7	11.2	28.4	
alfalfa	starch	20.6	12.9	25.6	9.4	31.8	
corn	plus	21.8	10.5	34.2	10.3	55.7	
sorghum	urea	86.9	6.3	84.7	5.9	83.9	
soybean		12.9	11.5	16.4	11.6	28.8	
alfalfa		22.5	10.8	34.0	8.2	28.1	
corn	none	28.2	10.7	28.9	10.8	58.4	
sorghum		55.6	7.5	49.0	7.1	65.2	

[a]Each number is the average percentage of wheatgrass plants diseased fou per pot.

[b]Each number is the mean oven dry weight per plant.

ın plant weights four weeks after sowing wheatgrass seed in soil
/e weeks from field plots previously amended with soybean,
nended with green soybean tops, tops plus roots, roots, and

	WEEKS AFTER INCORPORATION					
	THREE		FOUR		FIVE	
ːan wt. mg	dis. %	mean wt. mg	dis. %	mean wt. mg	dis. %	mean wt. mg
12.7	17.5	10.4	17.7	12.7	18.6	13.8
10.9	24.9	7.8	21.3	10.4	24.5	11.1
10.4	14.5	10.3	23.8	9.4	16.0	10.4
9.3	48.7	8.9	61.3	8.0	45.7	6.8
11.0	19.4	11.1	16.7	13.1	17.6	11.8
12.0	25.2	9.8	27.5	9.1	18.1	11.4
10.9	28.1	8.8	57.5	7.6	26.8	12.0
11.0	52.8	8.1	48.7	7.3	64.8	6.4
10.0	35.6	10.7	31.2	10.5	28.0	11.1
6.5	34.5	8.4	19.2	9.5	27.6	12.1
10.7	54.4	6.7	28.0	8.0	23.2	10.6
6.0	71.6	5.8	55.9	6.2	35.9	6.6
11.2	28.4	9.4	35.0	8.6	18.7	10.8
9.4	31.8	8.6	39.2	7.9	20.8	8.4
10.3	55.7	6.3	32.0	10.8	28.2	7.5
5.9	83.9	5.1	83.8	5.3	50.6	5.9
11.6	28.8	11.6	26.4	10.4	26.5	11.4
8.2	28.1	11.5	40.5	8.2	36.4	12.0
10.8	58.4	7.8	56.6	8.3	44.7	9.7
7.1	65.2	6.4	53.2	6.2	58.4	6.8

ꜱ plants diseased four weeks after sowing in three pots, 30 seed

DISCUSSION AND CONCLUSIONS

The results presented indicate that growing a particular crop in soil and/or incorporating its residue into the soil exerts a specific effect on the disease inciting potential of a particular root pathogen (in this instance *Pythium graminicolum*). Richardson (1942) demonstrated that soybeans as a previous crop and soil amendment produced healthier corn root systems than did red clover or rye. Such results were not only more or less duplicated here with the crested wheatgrass indicator plant, but were elaborated by counts of dead and diseased plants.

It was apparent from isolation results that large numbers of dead and diseased wheatgrass plants grown in sorghum soil were primarily due to an abundance and/or activity of *P. graminicolum*; conversely, that relative lack of dead and diseased plants grown in soybean soil was due in large part to relative scarcity or inactivity of *P. graminicolum*. This is in accordance with findings of Buchholtz (1949), Knaphus and Buchholtz (1958) and Harper (1960) who frequently recovered *P. graminicolum* from roots of diseased wheatgrass plants and infrequently from healthy appearing plants. In these experiments, therefore, the large disease inciting potentials which resulted from a crop of sorghum and the low potentials which resulted from a crop of soybeans are expressions of relative abundance and/or activity of this particular pathogen from the presence of these crops growing in the soil.

The data are not conclusive, but a definite change--lowering by soybeans, increasing by sorghum--in disease inciting potential is clearly indicated. The level for corn can be assumed to be a point of reference. Several years of continuous cropping to corn preceded the four crops in this experiment. An additional year of corn might be expected to not have much additional "corn effect". Furthermore, greenhouse assays of soil from the several plots indicated no significant differences among plots before and up to four weeks after the four crops were planted. But in subsequent assays, greenhouse and field, "sorghum soil" was above and "soybean soil" tended to be below "corn soil" in disease inciting potential. The conclusion that sorghum increased and soybeans decreased the disease inciting potential by P. graminicolum seems inescapable.

Sorghum is a host of P. graminicolum (Middleton 1943), whereas soybean is not (Knaphus 1960); corn (Middleton 1943) is a host, but alfalfa* is not. Whether or not sorghum is much more susceptible than corn has not been determined. Since both soybean and alfalfa apparently are immune to P. graminicolum, it is of interest that a soybean

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*Buchholtz, W. F., Ames, Iowa. Susceptibility of alfalfa to P. graminicolum. Private communication. 1961.

crop seemingly reduced disease inciting potential more than alfalfa. It also is noteworthy that alfalfa, a non-host, produced an effect similar to corn, a host. Although the differences were not significant in most instances, disease inciting potentials in alfalfa plots tended to be lower than in corn plots.

In this dissertation disease inciting potential was shown to be associated with abundance and/or activity of *P. graminicolum*, but the approach and methods were directed toward determining whether or not this pathogen was involved. However, the techniques used were not such as to definitely exclude all other root pathogens or perhaps other mitigating factors.

SUMMARY

An experiment during the 1960 growing season indicated that root systems of crop plants may modify the *Pythium graminicolum* disease inciting potential of naturally infested field soil. Soil samples taken from near roots of soybean, alfalfa, corn and sorghum plants at two week intervals during the months of June and July were assayed for their disease inciting potential by sowing an indicator plant (crested wheatgrass) in the soil samples and recording the percentages of plants diseased and relative frequency of isolation of *P. graminicolum* from the plant roots.

Significantly greater percentages of wheatgrass plants were diseased when grown in soil samples collected from near roots of sorghum than in soil taken from soybean, alfalfa or corn plots. Although isolation results were not so consistent as to be statistically significant, isolations from roots of wheatgrass grown in sorghum plots yielded *P. graminicolum* more frequently than did isolations from plants grown in soil from the soybean, alfalfa and corn plots.

In November of 1960, stem residues of the two legume and two grass crops were incorporated into the soil in the plots in which they had been grown. During the following growing season (1961) the *P. graminicolum* disease inciting potentials in the respective amended plots were determined by

greenhouse and field assays. There were small or no differences among percentages of diseased wheatgrass plants grown in soil samples collected early in the growing season (April 25) from the soybean, alfalfa, corn and sorghum residue amended plots. Similar comparisons for soil samples taken May 25, however, showed that there were significantly larger percentages of diseased plants in soil from the sorghum plots than in soil from the soybean, alfalfa or corn plots. Furthermore, soil from corn plots produced significantly larger percentages of diseased plants than did soil from soybean plots. Although the difference was not statistically significant, the percentage of diseased plants produced in soil from soybean plots was somewhat smaller (17.6 per cent) than in soil samples from alfalfa plots (32.9 per cent).

Analysis of root isolation data for the soil samples collected on April 25 showed that significantly more cultures of _P. graminicolum_ were obtained from roots of wheatgrass plants grown in soil from sorghum plots than from those grown in soil from the soybean plots. Similar comparisons for the soil samples taken May 25 revealed significantly larger percentages of recovery of _P. graminicolum_ from plants in soil from the sorghum plots than in soil from soybean, alfalfa or corn amended plots. Although not statistically significant, percentages of isolations yielding _P. graminicolum_ from plants grown in soil from soybean plots (26.3 per cent) were less than recovery from plants grown in soil from alfalfa and corn plots (40.3 and 43.8 per cent respectively).

Field assays of the P. graminicolum disease inciting potentials of the residue amended plots were as follows: wheatgrass seed was planted April 30, May 15 and June 5 in sections of each plot. Plant mortality was recorded one, two, three and four weeks following the week of maximum wheatgrass emergence. For the April 30 and May 15 plantings, plant mortality was least in soybean amended plots, intermediate in alfalfa and corn plots and largest in sorghum treated plots. For the June 5 planting, however, there were small or no differences in plant mortality among the four crop residue treated plots.

Wheatgrass plants were taken from the residue amended field plots four, five, six and seven weeks after planting. Root sections placed in water agar readily yielded P. graminicolum. Plants grown in sorghum amended plots consistently yielded the largest percentages of P. graminicolum regardless of planting date. For the first planting, isolations from plants in soybean and alfalfa plots yielded approximately equal numbers of P. graminicolum, and less than isolations from plants grown in corn plots. However, comparisons of P. graminicolum recovery data from plants of the May 15 and June 5 plantings in the soybean, alfalfa and corn plots showed little difference in percentages of cultures yielding this fungus.

Wheatgrass plants removed from the residue amended field plots and not utilized in the above isolation procedures were dried at 100 °C for 12 hours, and the oven dry weight per plant produced was calculated. The heaviest plants occurred in the soybean plots regardless of the planting date. Plants of intermediate weight were produced in alfalfa and corn plots and the lightest plants occurred in sorghum plots at the first and second planting dates. In general plants from the third planting date grown in alfalfa, corn and sorghum plots were of approximately equal weights.

Of the four crops used in the foregoing experiments, soybean crops and mature straw amendments appeared to induce a suppressive effect on the *P. graminicolum* disease inciting potential of naturally infested field soils. An attempt was made, therefore, to modify the disease inciting potentials of soybean, alfalfa, corn and sorghum plots late in the 1961 growing season by various soybean green manurial treatments. Tops, tops plus roots, and roots of young soybean plants were chopped up and incorporated in sections of the soybean, alfalfa, corn and sorghum plots. In addition, to a section was added potato starch plus urea and a section was nontreated to serve as a check. Greenhouse plantings of crested wheatgrass in soil samples collected at weekly intervals for five weeks from the above sections of plots showed the following: soybean tops and tops plus roots amendments tended to decrease the percentages of diseased wheatgrass plants as well as to increase the mean plant weights, regardless of the previous crop incorporation. The decrease in disease potential was most apparent in soil from those plots previously amended with sorghum residue. However, the beneficial effects of the soybean tops and tops plus roots residues in the sorghum plots was of short duration, no longer being apparent in soil samples from the fourth and fifth collections.

Soybean root amendments tended to increase the disease potential in all plots, regardless of the previous treatment. This increase was most apparent in soil samples collected one and two weeks after soybean root incorporation.

Starch plus urea treatment increased the disease potential of soil previously amended with sorghum. However, no such consistent effects of starch and urea were noted in plots previously amended with soybean, alfalfa or corn residues.

Of the two legume and two grass crops used in the foregoing experiments, soybean crops and mature straw amendments appreciably decreased the _P. graminicolum_ disease inciting potential of naturally infested field plots. Green manure of soybean tops induced a similar effect in that a decrease in disease potential was observed following the green manurial treatment. On the other hand, field plots planted to and amended with sorghum greatly increased the _P._

graminicolum disease potential of the treated soil. Field plots treated as above with corn and alfalfa crops demonstrated an intermediate disease potential between those of soybean and sorghum.

Deterrent "soybean effects" described above were probably not a manifestation of plant response to an increase in available nutrients, but rather a decrease in abundance of and/or activity of *P. graminicolum* in the soil of the treated plots. This conclusion is substantiated by the starch plus urea treatments in the sorghum plots (i.e. an apparent increase in disease potential following the starch plus urea amendment).

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